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MICRO-MORPHOLOGICAL STUDIES OF TWO SHRUB SPECIES *AGERATINA ADENOPHORA* (R. M. KING & H. ROB) AND *LANTANA CAMARA* L. (SENSU LATO) AFFECTED BY AIR POLLUTION

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ABSTRACT

Air pollution has become a serious environmental concern which is a major problem of Kathmandu Valley. The plants growing along the roadsides of the Kathmandu are under stress. The dust depositions on the leaves of shrubs were adversely affected by air pollution. It was found that dust particles affect leaf biochemical parameters which have changes micro-morphological symptoms. The study deals visible changes in the micro-morphological structure like specific leaf area, size of stomata, the thickness of epidermis and cuticle of leaves of Ageratina adenophora and Lantana camara investigated under polluted and control site (relatively less polluted area) of Kathmandu valley. Results showed that the plant species growing in a polluted site exhibited the significant reduction in the size of stomata, a thickness of the epidermal layer, a thickness of cuticle and the specific leaf area but the density of stomata increases in the polluted site as compared to the control site. Reduction in various parameters of two shrub species studied at three sites clearly indicates the deleterious effect of air pollution on plant health. It is concluded that vehicular emission had a significant effect on micro-morphological changes.

***KEYWORDS**: Cuticle Thickness, Epidermal Thickness, Stomata Density, Specific Leaf Area, Road side Pollution & Vehicular Emission*

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Original Article

INTRODUCTION

Plants are the source of life for all living beings, and they carry out many functions at the places where they exist (Yigit, 2016). Plant species, predominantly trees, and shrubs are important sinks for trapping and absorbing many gases, particulates, aerosols and airborne pollutants (Gajghate and Hasan, 1999) which helps in improving the quality of urban life (Powe and Willis, 2004). Air pollutants cause adverse effects on plants normal growth and development (Qadir and Iqbal, 1991). Various researchers studies have shown air pollution have the adversely affect on morphology, physiological and biochemistry changes of plants (Inamdar and Chaudhari, 1984; Iqbal, 1985, Gupta and Ghouse, 1988; Kulshreshtha et al., 1994; Pal, et al., 2000; Saadabi, 2011; Sharma and Roy, 1995). It was found that pollutant was responsible for leaf injury, stomata damage, premature senescence, decrease photosynthetic activity, disturbs membrane permeability and reduces the growth. (Tiwari et al, 2006; Horaginamani and Ravichandran, 2010). Environmental stress caused by air pollution is the most limiting factor for plant productivity and survivorship (woo et al., 2007). The reduced leaf area results in a reduced absorbed

radiation and subsequently in reduced photosynthetic rate. Several studies (Shan et al., 1996) suggest that low concentration of air pollutant may influence stomata indirectly through damage to the subsidiary cells or the epidermal cells that change the cell wall structure. Leaves indicate temporary pollution (Bass and Bauch, 1986; Fritts, 1976), which are the most visible and easily observable change occur in the micro-morphological parameters of the plant like change in the trichome density, stomata frequency and density, thickness of cuticle and toughness of epidermal cells are some of the associated changes (Pale et al., 2000). Thus plants are very sensitive to pollutions which can be used as indicators of air pollution.

Air pollution has become a serious environmental concern which is a major problem of Kathmandu Valley. Rapid urbanization, industrialization, poor maintenance of the road, increase in human population, increase vehicle deteriorates the air quality in Kathmandu valley. (Shrestha et al.2000; Pudasainee et al., 2010; Chen et al., 2015). The investigation has been carried out on different places of Kathmandu valley on the pollution load area in the air containing various pollutants like sulfur dioxide, oxide of nitrogen, hydrocarbon, and particulates The aim of this research is to compare micro-morphological and structural changes in invasive weeds of two shrubs *Ageratina adenophora and Lantana camara* in polluted and control sites of Kathmandu valley.

MATERIALS AND METHODS

Study Site

Kathmandu valley is situated in the middle part of the Himalayan range, at an altitude ranging from 1300-1400m above the sea level. It is located between latitudes 27.34^0 N and longitudes 85.11^0E and 85.32^0E. Kathmandu lies at a height of approximately 1300 meters, while the surrounding mountains range from 1500 meters to 2800 meters in height (Hildebrandt and Pokhrel, 2002). The valley is a bowl like in shape surrounded by mountains with a couple of hills above 2700 m elevation. The climate is considered temperate but it is influenced by the tropical monsoon. The study sites were selected on the basis of vehicular movement and the population density. The investigated sites taken were Narayanthan (C), Lainchour(P1), Basundhara(P2), and Dhumbarahi(P3). Narayanthan (C) was taken as control site whereas rests of the site were considered as experimental sites (See in figure 1). In these areas, the investigated plants grow in nature and are constantly exposed to tremendous vehicular pollutants.

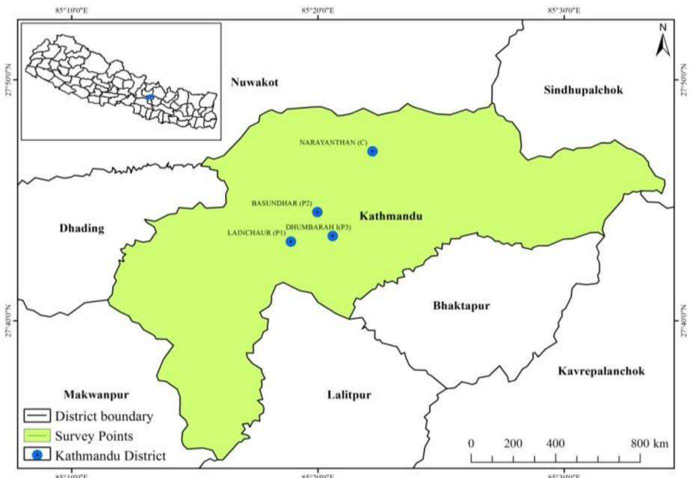

Figure 1: Study Area

Sample Collection

Roadside plant species like *Ageratina adenophora and Lantana camara* were taken from a polluted site (Basundhar, Lainchour, Dhumbarahi) and control site from Narayanthan. Ten broad leaves of largest size from each plant of selected site was collected and kept in polythene bags and brought to the laboratory. The loose dust particles on leaf surface were removed using a fine brush. Micro-morphological parameters like stomatal size, stomatal density, epidermal layer thickness, and cuticle were analyzed. For specific leaf area, ten leaves of dry weight from each site were taken. All micro-morphological parameters were photographed and measured under image J software program. The leaf epidermal peeled and slides were made by the method of lasting impressions technique (Rai and Mishra, 2013). In this method, one square cm of a leaf was painted by a thick patch of clear nail polish. The nail polish was allowed to dry completely and then nail polish was peeled out with the help of a clear cellophane tape. The leaf impression was taped on a slide and was examined under 15xX45x magnifications by light microscopy using the stage-ocular micrometer.

Micrometry

For quantitative microscopy, calibration was done by removing the eyepiece from the microscope, the lens was unscrewed and in the ridge, the eyepiece micrometer was placed. The lens was then replaced. The stage micrometer was then placed on the stage of the microscope and focused with the eyepiece scale superimposed. The division of ocular eyepiece, which coincides with the division of stage micrometer, was noted. The calibration factor was calculated using the formula.

Each division of eyepiece micrometer = (No. of divisions of stage micrometer / No. of divisions of the ocular micrometer) X 10.

The readings for quantitative values such as size length and breadth of stomata, stomatal density, cuticle thickness, specific leave area were measured. Mean values and values of standard deviation for each parameter were calculated from these readings using IBM SPSS (version 20) software program and the readings were tabulated.

RESULTS

Number, length, breadth of stomata, epidermal layer, a thickness of cuticle and specific leaf area of *Ageratina and Lantana* growing at polluted and control site of Kathmandu valley are demonstrated in table 1-5 below. Density per 100 μm^2 of Stomata increased significantly ($P<0.05$) in leaves from all investigated sites than in control. Leaves from all investigated sites showed a greater increase in the number of stomata along with its decreased size than leaves from the control site. Similarly, cuticle thickness and epidermal thickness in all leaf samples collected from polluted sites reduced significantly. This study clearly shows changes in the micro-morphological characters of *Ageratina adenophora and Lantana camara* due to urban air pollution in the Kathmandu valley.

From table (1), it was noted that the highest length and breadth of the stomata of *Ageratina adenophora* was observed from Narayanthan whereas lowest length and breadth from Lainchour. The highest length and breadth of *Lantana camara* was observed from Narayanthan whereas the lowest length and breadth from Basundhara.

From table (2), the highest density of stomata per 100μm^2 of *Ageratina adenophora* and *Lantana camara* was observed in Basundhara whereas the lowest density was observed in Narayanthan. From figure (2), it was observed that the stomata from polluted sites were the small and compact shape and more in number but the stomata from control sites were

large, broad and less in number.

From table (3), the thickness of the epidermal layer of *Ageratina adenophora* was highest in Narayanthan whereas lowest was observed in Dhumbarahi and Lainchour. Similarly, a highest thickness of an epidermal layer of *Lantana camara* was observed in Narayanthan and lowest in Basundhara.

From table (4), the highest thickness of cuticle of *Ageratina adenophora* and *Lantana camara* was observed in Narayanthan whereas minimum thickness was observed in Lainchour respectively. Overall the thickness of the epidermal layer and cuticle was found decreases in the polluted area than the control sites (See in a figure, 3).

From table (5), the highest specific leaf area of *Ageratina adenophora* and *Lantana camara* was observed in Narayanthan whereas lowest specific leaf area was observed in Basundhara.

Table1: Length and Breadth of Stomata (µm).

Sites	*A.adenophora* (Length)	*A. adenophora* (Breadth)	*L. camar* (Length)	*L. camara*
Narayanthan	47.60 ± 4.166 (d)	31.34 ± 3.9 (c)	45.35 ± 4.42 (c)	32.23 ± 4.85 (c)
Dhumbarahi	39.88 ± 3.35 (c)	27 ± 2.9 (b)	37.66 ± 4.96 (b)	22.34 ± 2.77 (b)
Basundhara	37.11 ±4.9 (b)	26.66 ± 6.37 (b)	33.03 ± 4.22 (a)	15.67 ± 3.81 (a)
Lainchour	33.13 ± 4.86 (a)	24.27 ± 2.7 (a)	39.55 ± 6.5 (b)	21.82 ± 3.31 (b)

Table 2: Stomata Density 100 /µm^2

Sites	*A. adenophora*	*L. camara*
Narayanthan	0.0095 ± 0.00193 (a)	0.0085± 0.002 (a)
Dhumbarahi	0.0153 ± 0.00252 (b)	0.0174± 0.0024 (b)
Basundhara	0.0275 ± 0.00286 (c)	0.0295± 0.0035 (c)
Lainchour	0.0162 ± 0.00294 (d)	0.0168± 0.00382 (b)

Table 3: Thickness of Epidermis

Sites	*A. adenophora*	*L. camara*
Narayanthan	22.10 ± 5.27 (c)	24.971 ± 5.77 (c)
Dhumbarahi	16.59 ± 3.38 (a)	19.87 ± 5.87 (b)
Basundhara	19.43±4.65 (b)	13.49 ± 2.3 (a)
Lainchour	16.72±3.65 (a)	19.57 ± 6.20 (b)

Table 4: Cuticle Thickness

Sites	*A.adenophora*	*L. camara*
Narayanthan	14.91 ± 3.15 (c)	16.34 ± 2.49 (c)
Dhumbarahi	8.54 ± 1.9 (b)	8.47 ± 2.53 (a, b)
Basundhara	7.74 ± 1.3 (b)	9.47 ± 5.64 (b)
Lainchour	3.32 ± 3.32 (a)	7 ± 5.5 (a)

Table 5: Specific Leaf Area

Sites	*A. adenophora*	*L. camara*
Narayanthan	337.78 ± 50.83 (d)	301.871 ± 148.63 (c)
Dhumbarahi	170.09 ± 56.66 (b)	114.18 ±60.74 (a, b)
Basundhara	130.35 ± 22.55 (a)	92.48 ±38.165 (a)
Lainchour	237.20 ± 35.79 (c)	181.13 ±77.08 (b)

Mean value followed by the same alphabet in the vertical column indicates insignificant difference at ($P>0.05$) according to one way ANOVA followed by Duncan multiple range test.

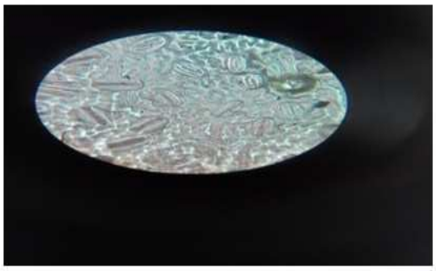

Figure A: Stomata of *A.adenophora* of Control Site

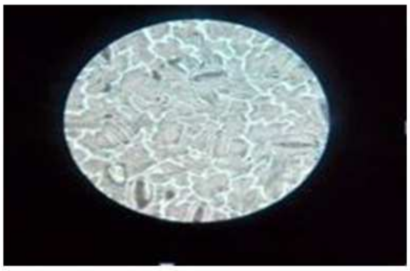

Figure B: Stomata of *A.adenophora* of Polluted Site

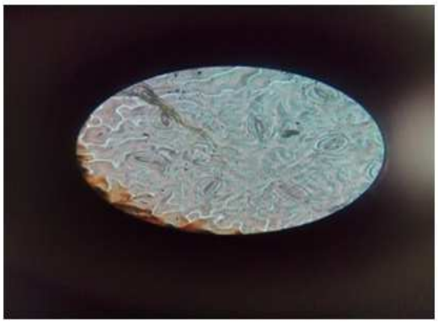

Figure C: Stomata of *L. camara* of Control Site

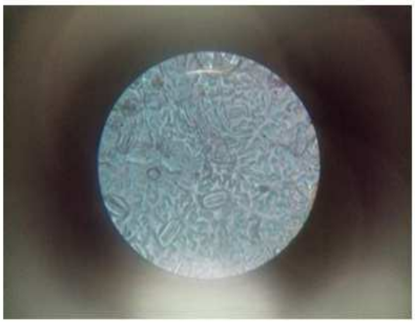

Figure D: Stomata of *L. camara* of Polluted Sites

Figure 2: Size and Density of Stomata (A-D)

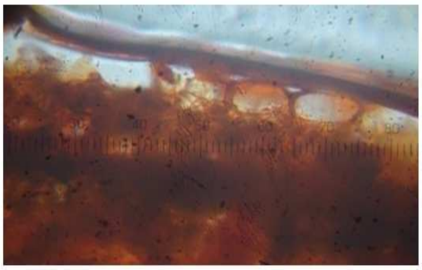

Figure A: Section of Leaf of *A adenophora* of Control Site

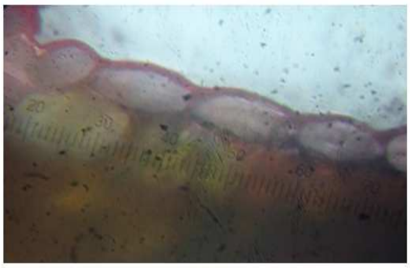

Figure B: Section of Leaf of *A adenophora* of Polluted Site

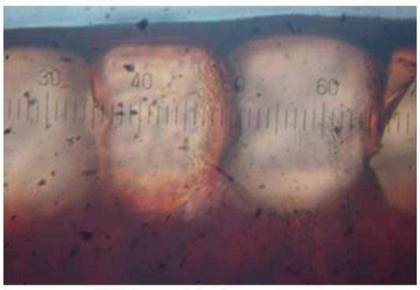

Figure A: Section of Leaf of *L. camara* of Control Sites

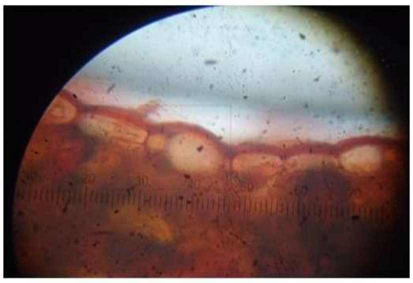

Figure B: Section of Leaf of *L. camara* of Polluted Sites

Figure 3: Thickness of Cuticle and Epidermal Layer (A-D)

DISCUSSIONS

The Kathmandu city is experiencing many environmental related issues due to automobile exhaust emissions. The deposition and settling of particulate matter on the aerial parts of roadside plants were increased due to automobile exhaust

emission resulting changes morphological symptoms. Leaves from all investigated sites showed the greater increase in a number of stomata with its decreased size of leaves than from the control site. In the polluted area stomata size of the plants were found decrease while the density was increased. Similarly, the thickness of an epidermal layer, cuticle, and specific leaf area decreases in the polluted area. One the similar study Kulshrestha et al., (2011) also reported significant differences in stomata features in the plant of the Syzygium cumini, Lantana camara, Nerium indiums growing in the area containing automobile exhaust pollutant. However, Rai and Mishra, 2013 has reported the reduction in a stomatal index of the *Calotropis procera, Croton bonplanduim baill and cannabis sativa* growing near a railway track. According to Tiwari, **(**2012) also recorded the changes in foliar morphology of two shrubs: Calotropis gigantia, Ipomoea fistulosa induced by air pollution. She also observed that the size of stomata was found to be reduced in both the species growing at polluted sites.

Different studies such as Jasminum sambac (kulshreshtha et al.., 1980), Calotropis gigantean (Ramanathan and Kanabiran, 1989), Azadirachta indica and Dalbergia sissoo (Sharma and Roy, 1995), Azadirachta indica and Polyalthia longifolia (Pale et al., 2000), Cassia siamea (Aggarwal, 2000), and Nyctanthese arbortristis, Quisqualis indica and Terminalia arjuna (Rai and Kul shreshtha, 2006) also shows similar results of reduction in the size of stomata and epidermal cells at polluted sites was observed. The accumulations of pollution in the plants have directly influenced by the source of pollution (Satyanarayana et al., 1990). However other studies on air pollution using lichens and mosses as biomonitoring reported that Naryanan are comparatively less polluted than other investigated sites (Chettri et al., 2001). The effect of particulate matter due to auto exhaust emission on plant growth growing roadside reported earlier in the literature around the world and very few studies have been done on this aspect in our the country.

A number of reports exposed that pollutant accumulation levels in plants are increased numbers of stomata have been reported to be a response to environmental stress, and seem to be an important strategy of controlling the leaf absorption of pollutants by plants (Gostin, 2009). The possibility of an increase in the number of stomata per unit area may be due to pollution stress. It is expected that because of pollution stress, normal growth of cells are restricted and that resulted in a smaller size. On the other hand, plants also need air to run the physiological processes and for that, they also need to prepare the number of stomata. The decrease in the size of stomata may also be an avoidance mechanism against the inhibitory effect of a pollutant on physiological activities such as photosynthesis (Verma et al., 2006).

CONCLUSIONS

The plants growing along the roadsides of the Kathmandu Valley are under stress. The dust depositions on the leaves of shrubs were adversely affected by air pollution. Leaf is directly damaged by the several types of pollutant released into the atmosphere through automobile exhaust. The study shows that change in the micro-morphological characters of *Ageratina adenophora and Lantana camara* was due to air pollution which can serve as an early warning system of the deteriorating quality of air. It was found that dust particles affect leaf biochemical parameters which have changes micro-morphological symptoms. Leaves from all investigated sites showed a greater increase in the number of stomata along with its decreased size than leaves from the control site. The size of stomata of plants in the polluted area was found to decrease while the density increased. Similarly, the thickness of the epidermal layer, cuticle, and specific leaf area decreases in the polluted area. These changes may be an adaptive response to the stress of air pollution. Thus characteristics studies of leaf area, size of stomata, a thickness of epidermis and cuticle of leaves can be considered good bioindicators for vehicular pollution studies. It is concluded that vehicular emission had a significant effect on micro-morphological changes.

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